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ANALYTICAL, CHARACTERIZATION, AND STABILITY STUDIES OF ORGANIC CHEMICALS, DRUGS, AND DRUG FORMULATION

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14. ABSTRACT

Although the overall purpose of this contract was to perform chemical/physical analyses on bulk pharmaceutical substances and formulated drug products of interest to the USAMRMC Drug Development Program for applications such as treatment of parasitic and infectious diseases and chemical/biological defense, the majority of time and effort during the first year was expended to select, award, and monitor three subcontractors to produce a second and larger batch of current good manufacturing practices (cGMP) artesunic acid (AS) IV drug product. As a part of the cGMP manufacture, a program of stability studies was maintained over the entire contract period to ensure the continued integrity of the drug in its clinical use. Because our manufacturing process involves batch API sterilization with ethylene oxide, a procedure that does not lend itself to large-scale manufacturing, we studied the feasibility of lyophilization of AS in t-butanol, a process that involves easy sterile filtration, precise unit vial filling, lyophilization, and facile automation. We demonstrated the method in principle, but were unable to remove the residual t-butanol to <0.5%. With additional research using more sophisticated equipment, an acceptable AS lyo product should be possible.

A modest synthesis program was carried out at the request of our COR. We prepared and fully characterized four very-high-purity 1,2,4-triazolidine derivatives and one high-purity quinazoline derivative. All were submitted to the COR.

In addition to the above activities, the core project team continued to serve all areas of the Army by performing chemical analyses when required. Reports on these analyses were submitted to the COR. Drug substances studied include paromomycin and gentamicin, pentostam, and 17α-ethynlestradiol-3-sulfate, sodium.

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FOREWORD

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PI – Signature May 21, 2014

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INTRODUCTION

This final report for Contract W81XWH-09-C-0022 covers the period from October 22, 2008 to May 21, 2014. The report summarizes studies performed and lists the compounds analyzed. The report also lists the personnel receiving pay from this effort and cites publications, meeting abstracts, and patents that resulted from this contract.

Under this contract SRI performed analytical, characterization, and stability studies of chemicals, drugs, and drug formulations. From October 2009 to March 2013, the work was monitored by Mr. William Y. Ellis, the Contracting Officer Representative (COR), Chief, Department of Chemical Information, Division of Experimental Therapeutics, Walter Reed Army Institute of Research (WRAIR); and from April 2013 to May 2014, the work was monitored by Major Daniel P. Erwin, PhD. (M) ASCP, COR, Chief, Chemical Information Systems, Experimental Therapeutics Branch, WRAIR.

The overall objective of this project is the operation of an analytical laboratory to determine the identity, purity, strength, quality, physical and chemical properties, and stability of bulk pharmaceutical substances and formulated drug products of interest to the USAMRMC Drug Development Program for applications such as treatment of parasitic and infectious diseases and chemical/biological defense. Specific objectives are to design, develop, validate, and execute methods to determine the following characteristics of candidate bulk pharmaceutical substances and formulated drugs:

- Identity, purity, and strength
- Stability
- Other physical and chemical characteristics, including weight variation, content uniformity, and other such compendial requirements
- Qualitative and quantitative identity of impurities
- Special projects not covered by the above characteristics.

FINAL REPORT

Activities Related to Artesunic Acid (Artesunate, AS)

Under this contract we devoted considerable effort and time to the selection and award of subcontractors to manufacture a second and larger batch (9,000 units) of artesunate (AS) parenteral drug product, WR256283, and its phosphate dissolution medium, WR135946, and to monitor the stability of the new batch as well as continuing to monitor the stability of the old batch.

Although our current process of preparing the AS parenteral drug product has been suitable for small-size batches, it is unsuitable for large-scale productions owing to its use of ethylene oxide for sterilization, which is done in small batches. The generally recognized method of choice to produce a parenteral drug product is lyophilization (lyo), which offers the advantages of easy/rapid sterile filtration, precise metered unit filling, gentle solvent removal, and facile automation. In general, the term lyophilization or freeze-drying refers to aqueous systems. For all practical purposes, AS is water insoluble, owing to its hydrophobic nature. However, AS contains a carboxylic acid function, which enables its ready dissolution in aqueous bases. Once in aqueous solution, however, the half-ester function in AS readily hydrolyzes to dihydroartemisinin (DHA), the pharmacologically active species that has limited solubility in aqueous media and falls out of solution. AS dissolves readily in aqueous basic media, its solutions freeze quickly, and its lyophilizations proceed smoothly, but its residual “cakes” cannot be obtained in anhydrous form, even under heroic conditions. The residual water in the lyophilizates continues to permit AS hydrolysis to DHA, which has limited solubility in the phosphate dissolution medium, and the resulting precipitate caused the USP <788> test (Particulate Matter in Injections) to fail. For this reason, we terminated our attempts to produce an aqueous AS lyo product.

Although much information on nonaqueous lyophilization is available in the open literature, much of it is academic in nature; no product produced by this means is commercially available. Our early attempts to prepare a nonaqueous “lyo” product through a subcontractor failed because of both the difficulty in removing the organic solvent and the glass-like quality of the residual AS (lyophilizate), which reconstituted unacceptably slowly.

A nonaqueous lyo drug product of an experimental nitrosourea anti-tumor agent has been reported. The nitrosourea was lyophilized from its solution in t-butyl alcohol (tBA); the residual solvent was reported to be <0.1%, and the resulting cake reconstituted readily. We have attempted to prepare a lyo product of AS using tBA. While sterilization by this approach should prove facile, complete removal of tBA has been only partially successful. The amounts of tBA remaining in our lyo cakes varied from one to several percent. With additional development using advanced lyo equipment, an acceptable lyo product should be possible.

Among the numerous tests required for product release and stability testing, the most difficult to perform is USP <788>. This test can be considered nonessential for determining stability of our AS drug product because its true causative failure should be the sample’s DHA content, which can be more readily determined with high specificity and accuracy using chromatography. This test is a part of the stability test program

because it was required for product release and was inadvertently carried over for stability testing. To pass USP <788>, the reconstituted solution must contain less than 6,000 counts of ≥10 μm particles per vial. Particles that are counted in our reconstituted solution include air bubbles, undissolved AS and DHA, lint, and other foreign matter. Foreign matter counts originating from the API itself and from the dissolution medium amounted to about 100 counts; these low counts have been essentially invariant and merit no further consideration. Counts from other sources, however, can easily cause test failure. After a great deal of development, including studies on rates of DHA formation and on apparent solubility of DHA in phosphate containing AS, along with rates of air bubble formation/dissipation, we finally settled on a sample preparation protocol that enables the reconstituted drug product to readily pass USP <788>.

Using our developed protocol, Dalton Pharma Services (Dalton) was able to release the clinical lot to the Army and to pass the stability testing for the first 24 months. The overall 2-year stability pattern of the Dalton-produced batch was essentially that found for the SRI-produced batch. When Dalton applied the proven procedure to the 36-month stability study samples, the results failed USP <788>. The determined DHA content in the 36-month stability sample was well within its solubility limit and could not have caused the USP <788> failure. SRI spent considerable time helping Dalton to adhere to the protocol until Dalton finally succeeded in passing USP <788>. At this point the 36-month stability of the clinical supply was established, enabling its continued use.

Activities Not Related to Artesunic Acid

SRI devoted much time and effort to preliminary development and preparation of experimental topical cream formulations on anti-leishmaniasis compounds. The compound studies included disulfiram, WR006058; two quinazoline derivatives, WR228275 and WR232155; two dinitroaniline derivatives, WR319713 and WR319769; and pseudomycin A, WR621183, and pseudomycin B, WR621184.

Another activity dealt with relatively simple formulations designed to increase drug plasma time without resorting to polymeric formulations designed for slow drug release. Following a patent awarded to Aida Salatinjants, we prepared and characterized a series of drug solutions and submitted them for plasma studies.

SRI also worked on the characterization of some strikingly large crystals isolated from leftover assay solutions of AS in phosphate. The highly crystalline material proved to be deoxyartemisin, which is the well-known artemisinin that has lost one of the oxygens from its endoperoxide. Its mechanism of formation has been well studied and reported, though not from solutions in phosphate under our mild conditions.

Still another major effort concerned chromatographic and spectral studies on WR621305, 17-α-ethynlestradiol-3-sulfate, sodium, which is difficult to obtain in pure, anhydrous state. We developed and applied useful nuclear magnetic resonance (NMR) methods for determining trimethylamine-sulfurtrioxide and trimethylammonium ion.

The change in COR in April 2013 soon brought a disruption of project activity by a stop-work order. A hiatus began about August 2013 and ended in November 2013 when we received a request for synthesis of four 1,2,4-triazolidine derivatives. SRI prepared, fully characterized, and submitted a 700-mg sample of very-high purity 1-phenyl-3,3-dimethyl-1,2,4-triazolidin-5-thione. In parallel, 500 mg of 1-phenyl-3-ethyl-3-methyl-

1,2,4-triazolidin-5-one, 230 mg of 1-phenyl-3,3-diethyl-1,2,4-triazolidin-5-thione, and 400 mg of 1-phenyl-3-ethyl-3-methyl-1,2,4-triazolidin-5-thione were prepared, characterized, and submitted. We received a second synthesis request for three quinazoline derivatives but, owing to time limitations, prepared only one. We synthesized and submitted a 13-gm sample of 6-iodoquinazolin-4(H)-one of approximately 95% purity.

Concurrent to all the activities already described, the contract core team continued to operate an analytical laboratory to serve all areas of the Army. Listed below are single or multiple samples of compounds/drug products analyzed, for which reports have been sent to the COR.

- WR001544, chloroquine phosphate
- WR002975, primaquine phosphate
- WR002977, amodiaquine HCl in combination drug ARSUAMOON
- WR006058, disulfuram
- WR100517, doxycycline hyclate
- WR035928, paromomycin sulfate
- WR073633, gentamicin sulfate
- WR142490, mefloquine HCl
- WR228275, a quinazoline derivative
- WR229870, stibogluconate
- WR232155, a quinazoline analogue
- WR252425, glucantime antimoniate
- WR256283, artesunic acid
- WR270295, pamaquine HCl
- WR279377, azithromycin
- WR279396, paromomycin/gentamicin
- WR282644, artelinic acid
- WR299958, decoquinate
- WR308275, cis-mirincamycin
- WR308276, trans-mirincamycin
- WR308338, paromomycin cream
- WR319713, a dinitroaniline derivative
- WR319769, a second dinitroaniline derivative
- WR621183, pseudomycin A
- WR621184, pseudomycin B
- WR621305, 17-α-ethynylestradiol-3-sulfate, sodium
- WR621361, 17-α-ethynylestradiol-3-sulfate, disodium
- WR773633, gentamicin base
- WR823412, ferroquine
- WR823413, a ferriquine metabolite

Presentations, Publications and Patents

No publications resulted from investigations conducted during the report period.

SRI presented “Analytical Studies on 17-α-ethynylestradiol-3-sulfate, sodium” at a SBL meeting in Washington, D.C., on March 12, 2012.

We have prepared a draft of a publication entitled “Chemical characterization of sodium stibogluconate (pentostam) in anti-leishmanial agent solution,” and a draft of a second publication entitled “Formation of deoxyartemisinin from artesunic acid in solutions of sodium phosphate”.

No patents were filed or awarded during the reporting period.

Personnel Receiving Major Contract Support

Personnel who received major contract support during the report period are:

- Peter Lim, P.I.
- Ronald Spanggord, Assistant P.I.
- Patrick Macauley, Chemist
- Jennifer Wang, Chemist
- Katherine Irwin, Chemist II

Subcontractors who received major contract support during the report period are:

- Dalton Pharma Services
- Afton Scientific Corp
- Steris Isomedix

SUMMARY/CONCLUSION

The most significant accomplishment achieved during the current contract period was the selection, award, and monitoring of a cGMP manufacturing, and stability maintenance of an intravenous dosage formulation of artesunic acid (AS). Over 9,000 units of the two-component dosage form was produced, released, and delivered to the Army. Integrity of the new, as well as the old, batch of AS IV drug product has been assured by at least 4 years of stability information.

Numerous topical cream preparations were developed and prototype units prepared for testing.

In addition, we successfully synthesized four very-high-purity samples of 1,2,4-triazolidine derivatives and one high-purity sample of 6-iodoquinazoline.

In conducting the above assignments we did not neglect the overall project objective of operating our analytical laboratory to determine the identity, purity, strength, quality, physical and chemical properties, and stability of bulk pharmaceutical substances and formulated drug products of interest to the USAMRMC drug development program for treatment of parasitic and infectious diseases, and for chemical/biological defense studies. Throughout the contract period, this laboratory continued to serve as a core resource to other areas of the Army when called upon.

Respectfully submitted,

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